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IS 15121 (2002): Animal Feeding Stuffs - Determination of the Contents of Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Sodium, and Zinc - Method Using Atomic Absorption Spectrometry [FAD 5: Livestock Feeds, Equipment and Systems]



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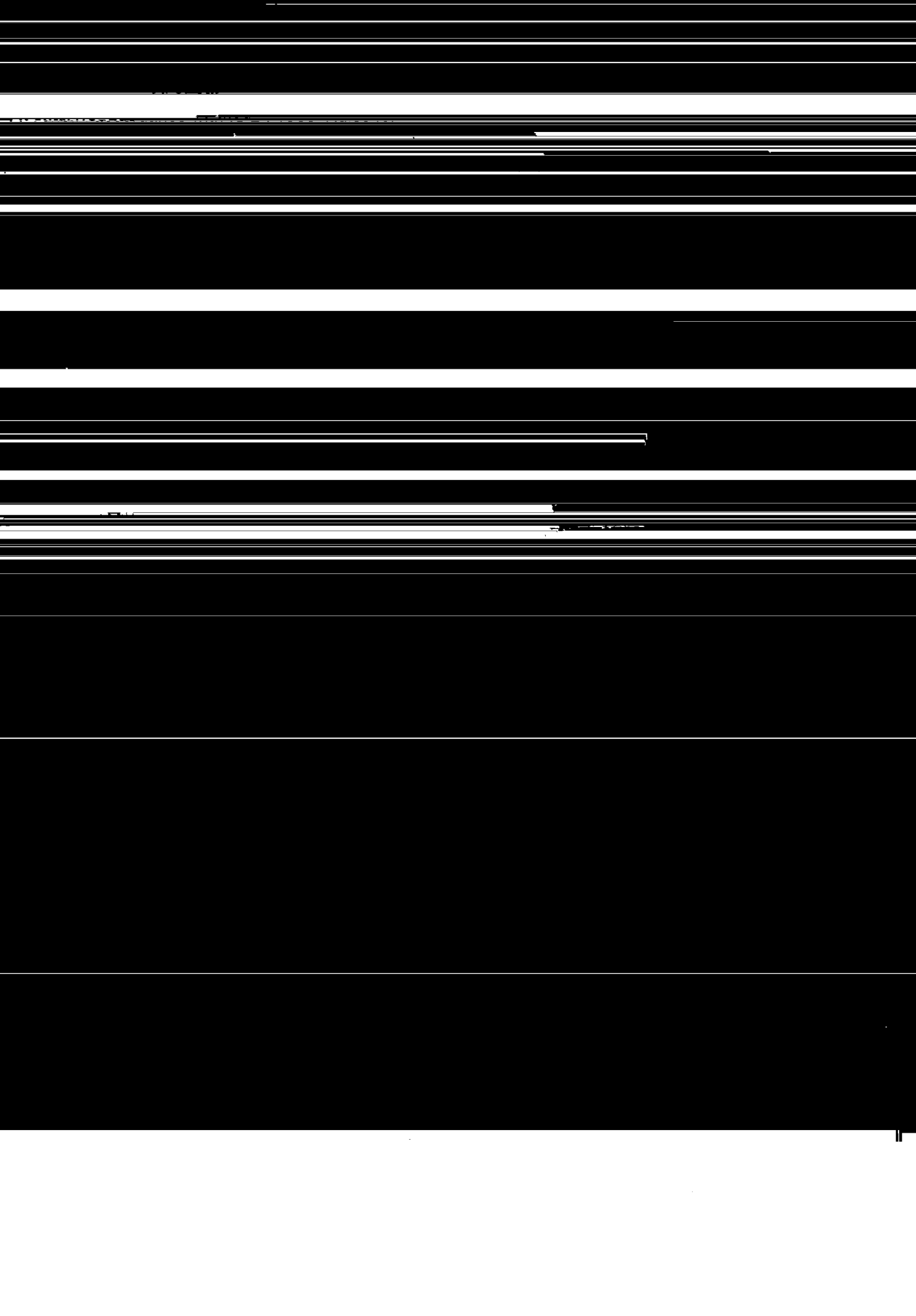
*Indian Standard*

**ANIMAL FEEDING STUFFS — DETERMINATION OF  
THE CONTENTS OF CALCIUM, COPPER, IRON,  
MAGNESIUM, MANGANESE, POTASSIUM, SODIUM  
AND ZINC — METHOD USING ATOMIC ABSORPTION  
SPECTROMETRY**

ICS 65.120

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## *Indian Standard*

# ANIMAL FEEDING STUFFS — DETERMINATION OF THE CONTENTS OF CALCIUM, COPPER, IRON, MAGNESIUM, MANGANESE, POTASSIUM, SODIUM AND ZINC — METHOD USING ATOMIC ABSORPTION SPECTROMETRY

## 1 Scope

This International Standard specifies an atomic absorption spectrometric method for the determination of the contents of calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), potassium (K), sodium (Na) and zinc (Zn) in animal feeding stuffs.

The method is applicable to all animal feeding stuffs.

The limit of determination for the elements concerned is as follows:

K and Na	500 mg/kg;
Ca and Mg	50 mg/kg;
Cu, Fe, Mn and Zn	5 mg/kg.

## 2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative documents referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*.

ISO 6498, *Animal feeding stuffs — Preparation of test samples*.

## 3 Principle

A test portion is dissolved in hydrochloric acid, if necessary after ashing in a muffle furnace at  $(550 \pm 15)$  °C. Any silica compounds present are removed by precipitation and filtration. The precipitate is dissolved in hydrochloric acid and diluted to the desired volume, then aspirated into the air-acetylene flame of the atomic absorption spectrometer.

The absorbance of each element is measured by comparison with the absorbance of calibration solutions for the same element.

## 4 Reagents and materials

Use only reagents of recognized analytical grade.

4.1 **Water**, complying with at least grade 3 in accordance with ISO 3696.

**4.2 Concentrated hydrochloric acid,**  $c(\text{HCl}) = 12 \text{ mol/l}$  ( $\rho = 1,19 \text{ g/ml}$ ).

**4.3 Hydrochloric acid,**  $c(\text{HCl}) = 6 \text{ mol/l}$ .

**4.4 Dilute hydrochloric acid,**  $c(\text{HCl}) = 0,6 \text{ mol/l}$ .

**4.5 Lanthanum nitrate solution.**

Dissolve 133 g of  $\text{La}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$  in 1 litre of water (4.1).

Another lanthanum salt may be used if the lanthanum content of the prepared solution is the same.

**4.6 Caesium chloride solution.**

Dissolve 100 g of CsCl in 1 litre of water (4.1).

Another caesium salt may be used if the caesium content of the obtained solution is the same.

**4.7 Stock solution of Cu, Fe, Mn and Zn.**

Mix 100 ml of water (4.1) and 125 ml of concentrated hydrochloric acid (4.2) in a 1 litre volumetric flask.

Weigh out the following:

- 392,9 mg of copper(II) sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ );
- 702,2 mg of ammonium iron(II) sulfate hexahydrate [ $(\text{NH}_4)_2\text{SO}_4 \cdot \text{FeSO}_4 \cdot 6\text{H}_2\text{O}$ ];
- 307,7 mg of manganese sulfate monohydrate ( $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ );
- 439,8 mg of zinc sulfate heptahydrate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ).

Transfer the weighed salts to the volumetric flask and dissolve them in water (4.1). Dilute to the mark with water.

The contents of Cu, Fe, Mn and Zn of this stock solution are each 100  $\mu\text{g/ml}$ .

NOTE Ready-prepared commercially available solutions may be used.

**4.8 Standard solution of Cu, Fe, Mn and Zn.**

Dilute 20,0 ml of the stock solution (4.7) with water (4.1) to 100 ml in a volumetric flask.

The contents of Cu, Fe, Mn and Zn of this solution are each 20  $\mu\text{g/ml}$ .

Prepare the solution fresh on the day of use.

**4.9 Stock solution of Ca, K, Mg and Na.**

Weigh out the following:

- 1,907 g of potassium chloride (KCl);
- 2,028 g of magnesium sulfate heptahydrate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ );
- 2,542 g of sodium chloride (NaCl).

Transfer the weighed salts to a 1 litre volumetric flask.

Add 50 ml of hydrochloric acid (4.3) to a beaker. Weigh into the beaker 2,497 g of calcium carbonate ( $\text{CaCO}_3$ ).

**CAUTION: Beware of the development of carbon dioxide.**

Boil for 5 min on an electric hot plate (5.4). Cool and transfer the solution to the volumetric flask containing the weighed salts of K, Mg and Na. Dissolve the salts and dilute to the mark with dilute hydrochloric acid (4.4).

The contents of Ca, K and Na of this solution are each 1 mg/ml; the content of Mg of the solution is 200  $\mu\text{g/ml}$ .

NOTE Ready-prepared commercially available solutions may be used.

#### 4.10 Standard solution of Ca, K, Mg and Na.

Dilute 25,0 ml of stock solution (4.9) with dilute hydrochloric acid (4.4) to 250 ml in a volumetric flask.

The contents of Ca, K and Na of this solution are each 100  $\mu\text{g/ml}$ ; the content of Mg of the solution is 20  $\mu\text{g/ml}$ .

Prepare the solution fresh in the week of use and store it in a polyethylene bottle.

#### 4.11 Lanthanum/caesium blank solution

Add 5 ml of lanthanum nitrate solution (4.5), 5 ml of caesium chloride solution (4.6) and 5 ml of hydrochloric acid (4.3) to a 100 ml volumetric flask. Dilute to the mark with water (4.1).

## 5 Apparatus

Rinse all volumetric ware, including the pipettes used for preparing calibration solutions, with dilute hydrochloric acid (4.4) before use.

If dedicated incineration dishes and glassware are used, it is not necessary to boil with hydrochloric acid each time before use.

Usual laboratory apparatus and, in particular, the following.

**5.1 Analytical balance**, capable of weighing to the nearest 0,1 mg.

**5.2 Incineration dishes** of platinum, quartz or porcelain, free from potassium and sodium, with a smooth unetched inner surface, upper internal diameter 4 cm to 6 cm, lower internal diameter 2 cm to 2,5 cm, and a height of about 5 cm. Before use, boil with hydrochloric acid (4.3).

**5.3 Glassware** of hard borosilicate glass.

Before use, boil with hydrochloric acid (4.3) and rinse with water.

**5.4 Electric hot plate or gas burner.**

**5.5 Boiling water bath.**

**5.6 Electric muffle furnace**, capable of being maintained at  $(550 \pm 15) ^\circ\text{C}$ .

**5.7 Atomic absorption spectrometer** (see ISO 6956 [6]), suitable for measuring at the wavelengths specified in 8.6.1 and 8.7.1, provided with an air-acetylene flame and a facility for correction for, or measurement of, background absorption.

**5.8 Hollow cathode lamps or electrode-less discharge lamps**, for the determination of Ca, Cu, Fe, K, Mg, Mn, Na or Zn.

**5.9 Filter paper** which does not release minerals.



## 6 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 6497 [5].

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Store the sample so that deterioration and other changes in composition are prevented.

## 7 Preparation of test sample

Prepare the test sample in accordance with ISO 6498.

## 8 Procedure

### 8.1 Detection of presence of organic matter

Heat a spatula with some test sample in a flame.

If the test sample melts without smoke, little organic matter is present.

If the test sample changes in colour and melting does not occur, the test sample contains organic matter.

### 8.2 Test portion

Depending on the expected content, weigh 1 g to 5 g of the prepared test sample (clause 7), to the nearest 1 mg, into an incineration dish (5.2).

If the test sample contains organic matter (see 8.1), proceed in accordance with 8.3.

If the test sample contains little or no organic matter (see 8.1), proceed in accordance with 8.4.

### 8.3 Dry ashing

Heat the incineration dish on a hot plate or over a gas burner (5.4) until the test portion has been completely carbonized. Avoid burning the test portion.

Transfer the dish to the muffle furnace (5.6) which has already been at a temperature of 550 °C for 15 min. Ash the sample for 3 h at this temperature.

Allow the sample to cool down then moisten the contents of the dish with 2 ml of water. If many carbon particles are present, dry the dish over the water bath (5.5).

Ash for another 2 h in the muffle furnace set at 550 °C.

Allow to cool down then add 2 ml of water.

### 8.4 Decomposition

While swirling, add 10 ml of hydrochloric acid (4.3), first dropwise until effervescence (possible development of carbon dioxide) has ceased, then faster. Swirl and heat the contents of the dish until almost dry. While drying, take care to avoid loss by splattering.

Dissolve the residue by heating with 5 ml of hydrochloric acid (4.3) and transfer the solution quantitatively with some 5 ml portions of water to a 50 ml volumetric flask.

Allow to cool, then dilute to the mark with water and mix. Allow the particles to settle and filter (see 5.9) the solution if it is not clear after 4 h.

## 8.5 Blank solution

Prepare for each measuring series a blank solution by carrying out the procedure according to 8.2, 8.3 and 8.4 without the test sample.

## 8.6 Determination of copper, iron, manganese and zinc

### 8.6.1 Measuring conditions

Adjust the atomic absorption spectrometer (5.7) in accordance with the manufacturer's instructions. Optimize the response of the instrument for measurement with the air-acetylene flame. For the determination of Cu, Fe, Mn and Zn set the following wavelengths:

Cu: 324,8 nm;

Fe: 248,3 nm;

Mn: 279,5 nm;

Zn: 213,8 nm.

### 8.6.2 Preparation of calibration curve

Prepare a series of appropriate calibration solutions by diluting the standard solution (4.8) with dilute hydrochloric acid (4.4).

Measure the absorbance of the hydrochloric acid (4.4). Measure the absorbance of the calibration solutions and subtract the absorbance measured for the hydrochloric acid (4.4).

Draw a calibration curve by plotting the corrected absorbances against the respective contents of Cu, Fe, Mn and Zn.

### 8.6.3 Measurement of test solution

Measure parallel to the calibration solutions, under identical circumstances, the absorbance of the test solution (8.4) and the blank solution (8.5). Subtract the latter absorbance from the first absorbance.

If necessary, dilute a quantity of the test solution and blank solution with dilute hydrochloric acid (4.4) to obtain an absorbance in the linear part of the calibration curve.

Proceed in accordance with clause 9.

## 8.7 Determination of calcium, magnesium, potassium and sodium

### 8.7.1 Measuring conditions

Adjust the atomic absorption spectrometer in accordance with the manufacturer's instructions. Optimize the response of the instrument for measurement with the air-acetylene flame. For the determination of Ca, K, Mg and Na set the following wavelengths:

Ca: 422,6 nm;

K: 766,5 nm;

Mg: 285,2 nm;

Na: 589,6 nm.

### 8.7.2 Preparation of calibration curve

Dilute the standard solution (4.10) with water (4.1). Add per 100 ml of diluted standard solution 5 ml of lanthanum nitrate solution (4.5), 5 ml of caesium chloride solution (4.6) and 5 ml of hydrochloric acid (4.3). Choose the dilutions so that appropriate calibration solutions are obtained.

Measure the absorbance of the lanthanum/caesium blank solution (4.11).

Measure the absorbance of the calibration solutions and subtract the absorbance measured for the lanthanum/caesium blank solution (4.11).

Draw a calibration curve by plotting the corrected absorbances against the respective contents of Ca, K, Mg and Na.

### 8.7.3 Measurement of test solution

Dilute a quantity of the test solution (8.4) and blank solution (8.5) with water (4.1). Add per 100 ml of diluted solution 5 ml of lanthanum nitrate solution (4.5), 5 ml of caesium chloride solution (4.6) and 5 ml of hydrochloric acid (4.3).

Measure parallel to the calibration solutions, under identical circumstances, the absorbance of the diluted test solution and the diluted blank solution. Subtract the latter absorbance from the first absorbance.

If necessary, dilute a quantity of the test solution and blank solution with lanthanum/caesium blank solution (4.11) to obtain an absorbance in the linear part of the calibration curve.

## 9 Expression of results

Calculate the content of each of the elements calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc starting from the calibration curve and taking into account the mass of the test portion and the dilutions applied.

Round the result in accordance with Table 1 and express the result in milligrams per kilogram or grams per kilogram.

Table 1 — Rounding of the calculated content

Content		Round to
from	to	
5 mg/kg	10 mg/kg	0,1 mg/kg
10 mg/kg	100 mg/kg	1 mg/kg
100 mg/kg	1 g/kg	10 mg/kg
1 g/kg	10 g/kg	100 mg/kg
10 g/kg	100 g/kg	1 g/kg

## 10 Precision

### 10.1 Interlaboratory tests

Details of interlaboratory tests on the precision of the method are given in annex A. The values derived from these tests may not be applicable to concentration ranges and matrices other than those given.

### 10.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases exceed the repeatability limit ( $r$ ) mentioned in or derived from Table 2 or Table 3.

Table 2 — Repeatability limit ( $r$ ) and reproducibility limit ( $R$ ) for premixes

Values in milligrams per kilogram

Element	Element content		$r$	$R$
	from	to		
Ca	3 000	300 000	$0,07 \times \bar{w}$	$0,20 \times \bar{w}$
Cu	200	20 000	$0,07 \times \bar{w}$	$0,13 \times \bar{w}$
Fe	500	30 000	$0,06 \times \bar{w}$	$0,21 \times \bar{w}$
K	2 500	30 000	$0,09 \times \bar{w}$	$0,26 \times \bar{w}$
Mg	1 000	100 000	$0,06 \times \bar{w}$	$0,14 \times \bar{w}$
Mn	150	15 000	$0,08 \times \bar{w}$	$0,28 \times \bar{w}$
Na	2 000	250 000	$0,09 \times \bar{w}$	$0,26 \times \bar{w}$
Zn	3 500	15 000	$0,08 \times \bar{w}$	$0,20 \times \bar{w}$

$\bar{w}$  is the mean of the two results, in milligrams per kilogram.

**Table 3 — Repeatability limit (*r*) and reproducibility limit (*R*) for animal feeds**

Values in milligrams per kilogram

Element	Element content		<i>r</i>	<i>R</i>
	from	to		
Ca	5 000	50 000	$0,07 \times \bar{w}$	$0,28 \times \bar{w}$
Cu	10	100	$0,27 \times \bar{w}$	$0,57 \times \bar{w}$
Cu	100	200	$0,09 \times \bar{w}$	$0,16 \times \bar{w}$
Fe	50	1 500	$0,08 \times \bar{w}$	$0,32 \times \bar{w}$
K	5 000	30 000	$0,09 \times \bar{w}$	$0,28 \times \bar{w}$
Mg	1 000	10 000	$0,06 \times \bar{w}$	$0,16 \times \bar{w}$
Mn	15	500	$0,06 \times \bar{w}$	$0,40 \times \bar{w}$
Na	1 000	6 000	$0,15 \times \bar{w}$	$0,23 \times \bar{w}$
Zn	25	500	$0,11 \times \bar{w}$	$0,19 \times \bar{w}$

$\bar{w}$  is the mean of the two results, in milligrams per kilogram.

**NOTE** In Table 2 and Table 3, the repeatability and reproducibility limits are given in the form of a formula for each element and for the indicated range. The factor in this formula is an average over the investigated samples in the indicated range. In exceptional cases higher values have been obtained for the determination of a specific element in specific samples. These samples have not been taken into account. Most likely cause of these deviations is lack of homogeneity of the samples concerned (see annex A).

### 10.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories by different operators using different equipment, will in not more than 5 % of cases exceed the reproducibility limit (*R*) mentioned in or derived from Table 2 or Table 3.

## 11 Test report

The test report shall specify:

- all information necessary for the complete identification of the sample;
- the sampling method used, if known;
- the test method used, with reference to this International Standard;
- all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- the test result obtained, or the two test results obtained if the repeatability has been checked.

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